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XLIV.—*Contributions to the Knowledge of the Physiology and Biology of the Protozoa.* By Dr. AUGUST GRUBER*.

Introduction.

In the following pages I propose to publish a series of experiments and observations which may furnish a contribution to the knowledge of the physiology of the Protozoa. The work is not a finished whole, and, above all things, not an exhaustive investigation; it is intended only to assist in furnishing materials for a structure which must still wait many years for its completion. A part of the facts contained in it I have already made public in preliminary communications †, and I repeat these here in a somewhat extended form. Other experiments, on the contrary, have not hitherto been published, and, indeed, have perhaps never previously been made. May both be received with some interest in scientific circles!

On artificial Divisibility and Regeneration in the Protozoa.

Experiments have already been made in former years upon artificial divisibility in the lowest organisms; for example, in

* Translated by W. S. Dallas, F.L.S., from a separate impression of the paper in the 'Berichte der naturforschenden Gesellschaft zu Freiburg i. B.,' Band i. (1886) Heft 2. Communicated by Dr. Wallich.

† "Ueber künstliche Theilung bei Infusorien," in Biologisches Centralblatt, Bd. iv. pp. 717 722, and Bd. v. pp. 137–141.

the last century, upon the great sun-animalcule, *Actinosphærium Eichhornii*, by its discoverer, Eichhorn himself; afterwards, namely in 1862, on the same object, by Häckel; Greeff, in 1867, artificially divided* the *Pelomyxa palustris*, which he described, and Häckel again made the same experiments with his *Myxastrum radians* †. They all succeeded in obtaining fragments capable of living on by the artificial division of these Protozoa. In the same way botanists have separated fragments from plant-cells, and indeed, as we shall have to notice hereafter, from multinucleate cells, and by this means have obtained small living individuals.

In ciliated Infusoria, and therefore in unicellular animals of complicated structure, these experiments were first made very recently, and indeed simultaneously, by M. Nussbaum and myself. Nussbaum, whose observations were published‡ before mine, operated with *Oxytricha*, and showed that if such an Infusorian was divided by a sharp cut longitudinally or transversely into two parts, these were able, within a short time, usually on the following day, to convert themselves again into perfect animals, each half replacing the other deficient one, the anterior end replacing the lost posterior end, and *vice versâ*; smaller fragments also were capable of completing themselves again.

For my part I have made use of another object in my experiments, namely, the large *Stentor cæruleus*, which certainly is not so resistent as *Oxytricha*, and cannot be preserved alive so long isolated; but, on the other hand, from its larger dimensions and its exceedingly characteristic mode of ciliation, allows the course of the regeneration to be more easily and distinctly watched §.

In the first place, as regards small injuries, these heal very rapidly, the cortical layer closing together at once over the wound; when the mutilations are more profound, on the contrary, the Stentors often acquire a crippled form, which is either not lost again or, as I have frequently observed, only disappears quite gradually. Thus a Stentor which had grown deformed in consequence of a cut on one side, and had become drawn out into an abnormal hinder extremity quite close to the peristome, occupied eight days before it had again become quite normal.

* "Ueber *Actinosphærium Eichhornii*, &c.," in Arch. für mikr. Anat. Bd. iii.

† "Monographie der Moneren," Jenaische Zeitschr. für Naturwiss. Bd. iv. (1868).

‡ Sitzungsb. der niederrh. Gesellsch. für Natur- und Heilkunde zu Bonn, Sitzung. der med. Sect. 15 Dec. 1884.

§ As previously stated (see Introduction) I have already made known what follows in shorter communications.

Stentor cœruleus is particularly well suited for the observation of the mode in which injuries to the external surface heal up again, on account of the broad blue stripes of the cortical layer. Thus if we make a short sharp cut in the cortical layer with the scalpel, the animal of course shrinks together and the wound at once closes, but at the same time the stripes and the muscular fibres are still separated by the cut at the place affected and can only gradually grow together again. In a few hours, however, this is also effected, but usually so that a displacement has taken place, the corresponding ends not having found each other, and furcations and bendings of the stripes are produced which always betray the place of the incision. The mobility of the Infusorian is, however, by no means affected by this, and indeed, even in the normal animal, furcations of the stripes and muscular fibres very often occur towards the anterior extremity. The mode in which the extremities of the cut muscular fibres endeavour to find each other and finally grow together again no doubt resembles on a small scale the course of events which we have to imagine in the process of wound-healing in the muscles of higher animals.

As regards the complete division of the Stentors into two or more parts, I have already remarked that this usually leads to the production of the same number of perfect Infusorians as there were pieces, although with a limitation, as will be shown hereafter. With some practice the section itself is easily performed, if a tolerably sharp little scalpel is employed, only it is often difficult to ascertain the right quantity of water; for if the drop on the slide is too large the Infusorian swims away from under the knife, while if it is too small the Stentor becomes too much flattened out and quickly deliquesces after the section is made. I may here mention, however, that the deliquescence may be prevented by quickly adding water, and that Stentors which have already suffered considerable loss of substance may recover and become perfectly regenerated.

If the section has been cleanly made and the quantity of water rightly adjusted, the two wounded surfaces immediately close again, and the two halves swim briskly about; they may be fished out with the pipette and isolated, which is best done in small watch-glasses, and then in the course of from twelve to twenty-four hours the lost parts are completely replaced in each of the pieces. If we employ a lens or a low power of the microscope the sections may be carried in any predetermined direction; and it then appears that the regeneration occurs most rapidly and completely when the section has gone transversely (fig. 1, p. 493), whilst with sections in the longitudinal line the two halves, which of course are long and narrow, usually become rolled up, and the regenerated parts often appear crippled at first, a peculiarity which, however, as already remarked, usually disappears subsequently. We may therefore say of *Stentor*, as of *Oxytricha*, *that the anterior end replaces the lost posterior end, and the right side the lost left side, and vice versâ.*

The question now is, in what manner does this regeneration take place? and for the settlement of this question *Stentor* is perhaps the best of all Infusoria. Let us first of all consider the anterior part of a Stentor separated by a transverse cut; it is at first broadly truncated at the cut surface (fig. 1), but gradually the body becomes drawn out in length posteriorly, the streaks taper off, and in this way the well-known tapering posterior end is again developed, in which the body-parenchyma protrudes as an apparatus of adhesion. In this mode of regeneration it would almost appear as if no new formation of parts occurred, but rather only a change of position of those already present. Of course the process of regeneration is much more complicated in those specimens of which the anterior end has been cut away transversely. In these also we have at first a straight line or flat surface at the point of section: in time, however, the body of the fragment becomes rounded off at its anterior end until it has again acquired a clavate form; but it is still destitute of any of the large cilia, as indeed the whole peristomial area, with the mouth and the spiral of cilia, has been removed. The reproduction of these lost organula actually takes place *in exactly the same way as in spontaneous division.* The latter process, as is well known, commences by the formation in the median line of the dividing Stentor of a vertically placed stria of large peristomial cilia (membranellæ), and as the process goes on the more does this line of cilia grow, gradually bending into an arc, until finally it forms a circlet of cilia, which constricts off the so-called peristomial area from the rest of the body. At the same time one end (the right) of the stria sinks spirally into the interior of the body, and thus forms the mouth and the œsophageal funnel. In the "decapitated" Stentor also the new peristomial cilia make their appearance on one side, arranged in a vertical line (fig. 2), which then during the further growth surrounds the anterior extremity and originates the peristomial area and the mouth. Here, therefore, we have the interesting fact *that the regeneration of the organula in the Infusoria follows the same course as their new-formation in spontaneous fission.* The to us unknown impulse which induces the animals to divide, and the irritation caused by the violent removal of a part of the body, are identical in their effects. In the regeneration of the lost organs and portions of tissue in the higher animals we have essentially the same phenomenon, only with this difference, that in the latter the cells perform what in the Infusoria is performed by the elementary particles, micellæ, or what we choose to call them. If we ascribe regeneration in the Metazoa to the influence of embryonally formed cells, *we must here award the function of new-formative elements to originally-formed micellæ*, which, as we shall see hereafter, are subject to the directing influence of the nucleus.

I believe that I can show in the case of *Stentor* that the process of regeneration is a regular one and homologous with the well-known process of new-formation in spontaneous fission. We might conceive that in every Infusorian at a certain time the materials for the organs of a new animal are prepared and stored up in the interior, and at the given moment begin to group themselves; in the artificial division of an Infusorian in this stage, and therefore ready for spontaneous division, a process, which would have occurred at this moment as the new formation preceding spontaneous reproduction, would appear to us to be regeneration. But this is not the case; for, in the first place, it is very improbable that all the Stentors employed in the experiments were precisely in the same stage of development; and, in the second place, I have often divided such as had just been produced by spontaneous fission, or were still engaged in that operation, and these have also become regenerated, which would not have been possible upon the above hypothesis, as the reserve-material in them would just have been used up. *Regeneration, therefore, can be due only to a conversion of elementary parts already present, taking place rapidly upon external irritation.*

As regards the *degree of the regenerative faculty, this is very high in* Stentor; *and no particular part of the body appears to be specially disposed thereto, but all parts of the body react in the same way.*

This is clear from the following experiments:—

If we cut away the extremity of a Stentor far behind the middle of the body, this extremity has the same regenerative faculty as one the cut surface of which was near the anterior end; or, further, if we divide a Stentor first of all by a longitudinal incision into right and left halves, and divide each of these two pieces again into an anterior and a posterior portion, or, which answers still better, make the transverse section first and the longitudinal ones afterwards (fig. 3), all the four divisions, although derived from quite different parts of the body, are equally able to become developed into perfect animals. There is, however, a difference in the mode of regeneration of such different fragments, because in those quadrants (if I may use the expression) which have retained a portion of the peristomial circlet of cilia, the deficiencies are made up by simple growth, whilst in the parts which show no peristomial cilia the latter must be formed anew in the way above described. There is also no more difficulty in dividing a Stentor into three pieces in such a manner as to obtain the anterior and posterior ends and a median section isolated from each other (fig. 4); this last is likewise able to become perfectly regenerated in the same time as the other fragments, although it has to form anew both the anterior and the posterior extremities.

Although these experiments distinctly prove the high regenerative faculty of the Stentors, the following one does this still more clearly:—A *Stentor cœruleus*, which I will indicate as A, was divided transversely into two halves; next day these had grown into two perfect animals B and B′; the anterior end was now separated from B, and B′ was again divided transversely, when it appeared, after the lapse of twenty-four hours, that B had again regenerated itself, and that the two halves of B′ had become developed into two perfect Infusoria, C and C′. B was again divided, but without result, as on the next day it had perished; while of the two divisional pairs into which I had again divided C and C′, only one derived from C had perished, and the two halves of C′ had again become regenerated into two small Stentors, D and D′; and, finally, I succeeded in obtaining artificially from D and D′ a generation E; but these individuals had now become so small that they had lost their vitality and soon perished. I had therefore succeeded in carrying out artificial division on the same objects for five consecutive days in which regeneration of the lost parts took place five times.

Nussbaum has also demonstrated that artificially multiplied Infusoria, under favourable conditions, are afterwards able to divide further spontaneously, and I have observed the same thing in my experiments. Thus, for example, on the 10th December I had transversely divided nine Stentors and only isolated the hinder extremities; on the next day all the nine had developed new, perfect peristomial areas with the ciliary circlet and mouth; on the 13th December one of these regenerated animals showed the commencement of spontaneous fission, and on the 15th the nine specimens had become fifteen, which I kept alive until the end of the month.

Another experiment is as follows:—A Stentor was, on the 28th April, divided transversely into two halves, both of which had become regenerated on the following day; on the 30th the two artificially produced daughter-individuals had, almost simultaneously, divided again spontaneously. In two other artificially separated divisions also, one of which had been at first deformed, natural fission occurred simultaneously, as also in a third experiment. We thus learn from observation the interesting fact that *two artificially produced halves are able to increase spontaneously at exactly the same time*, although after section they were apparently not equivalent, and the anterior portion, which still possessed the most complicated part of the body, the peristomial area with the mouth and œsophagus, really had only to go through the process of wound-healing, while the posterior portion must have produced all the above organs anew. Nevertheless it was able to answer to the impulse leading to fission just as quickly as the other. This also is a proof *that the material for new-formations in the Infusoria is not stored up predisposed as such, but that the elementary parts above indicated as primitive in the protoplasm are convertible at any time.* That the impulse to fission, which, as will be shown hereafter, we must seek in the nucleus, occurred simultaneously in the two separated portions cannot surprise us if we consider that the nuclear constituents present in them were in connexion only a little while before, and therefore must have agreed in their constitution and in their action upon the protoplasm.

I may mention, in conclusion, that regeneration can be produced also in parts which are not completely separated from each other, so as to form Stentors with two anterior or two posterior ends. Thus, for example, I had divided a Stentor by a longitudinal incision in such a way that one of the two halves, which were still connected behind, retained nearly the whole peristome and the other only a small part of it (fig. 5); the former immediately completed itself again, but in the case of the second half some days elapsed before it had again developed a perfect peristome with the mouth (fig. 6). Thus two perfect Stentors, only united at the base, were produced, and they further contained a connected chain of nuclear joints. Unfortunately I could not keep this pair of twins long alive, as the water in which I had isolated them became foul. In the same way we may succeed by means of incomplete longitudinal sections in producing animals which show two posterior ends attached to a common fore part. However, halves divided in this way do not always remain connected, but usually they tear themselves apart by twisting movements.

I have hitherto spoken exclusively of the regenerative faculty of *Stentor cæruleus*, and now comes the question as to how far this occurs also in other Infusoria. I have already mentioned Nussbaum's experiments, which prove that *Oxytricha* behaves in the same way; I have myself operated with *Stentor polymorphus* and with *Climacostomum virens*, in both of which the parts removed were replaced within twenty-four hours; in *Paramæcium* I also succeeded in removing the anterior end, isolating the posterior end, and finding it regenerating on the next day. On the other hand, there are other Infusoria which present difficulties that are frequently insurmountable. Thus Nussbaum could not keep artificially-divided *Opalinæ* alive, as no cicatrization of the wounded surface took place; ciliary action continued for an hour or two, but then the divided portions perished. With *Loxodes rostrum* experiments in artificial division were equally unsuccessful; these Infusoria usually deliquesce immediately after the section has been made, or if one succeeds in obtaining and isolating divided portions, these perish before any regeneration has taken place.

It is remarkable that *Opalina* and *Loxodes* in particular show themselves to be so little capable of regeneration, as they are both multinucleate, and, as I remarked at the beginning, experiments in artificial multiplication were first made successfully upon multinucleate Protozoa—*Myxastrum**, *Protomyxa*, and *Actinosphærium*.

I have also employed the great Holotrichous Infusorian *Cyrtostomum leucos* in experiments, and observed that in this also the regeneration does not take place so rapidly as in the above-mentioned Heterotricha, *Stentor* and *Climacostomum*; although a new mouth and œsophagus are formed, the body remains deformed for a long time. Even the Heterotricha do not all behave alike in this point, for I never succeeded in multiplying *Spirostomum*, for example, artificially, as it is very difficult to keep isolated in small quantities of water, and even when uninjured soon perishes. *Very probably these differences in the regenerative capacity of the Infusoria depend only on the greater or less faculty of existing under not quite natural conditions, and the power of replacing lost parts is, in my opinion, proper to all Protozoa, notwithstanding the above-cited negative results.*

But if we inquire *why* the Infusoria have so high a regene-

* *Myxastrum* was referred to the Monera by Häckel, as possessing no nucleus; but very probably the nuclei merely escaped his notice, for by the employment of our present methods of treatment they may easily be shown in *Myxastrum liguricum* (see Gruber, "Die Protozoen des Hafens von Genua," in Nova Acta Acad. Leop. Carol. Bd. xlvi. 4, p. 505).

rative power as we have demonstrated, for example, in *Stentor*, this question is not so easy to answer; for when living freely they will rarely have to suffer injuries, or at all events such injuries as we can inflict upon them artificially with the scalpel. In multicellular animals this is quite otherwise; in their case we know that they have very often lost parts of their bodies by violent attacks, and in their case we are not surprised that many of them are endowed with a very highly developed regenerative capacity, which has to play an important part in the preservation of the species. But what is the case in the Protozoa? In my preliminary communication I have already expressed the opinion that *perhaps the acquisition of the regenerative faculty by the Infusoria (and by the Protozoa in general) may depend on the fact that they frequently break up spontaneously into irregular fragments, and that then many of these fragments are able to become developed again into normal animals.*

This spontaneous breaking up is a phenomenon easy of observation in the life of the Infusoria, and one that I have already seen in a number of species; it struck me particularly in the case of a colony of *Oxytricha*, and in this, among the ruins which circulated in the water, I found many which were indeed much smaller than the normal animals, but still more or less regularly formed, so that we may assume that here a regeneration had taken place. I do not venture to draw any more certain conclusion, because at the time I had something else in view, and did not go into this point with sufficient exactness. In other Infusoria, however, the breaking up of the body into small fragments and the subsequent growth of these into normal animals is a regular phenomenon and the ordinary mode of increase, namely in the *Opalinæ*. It is remarkable, however, that these are precisely the Infusoria which, as already mentioned, could not be artificially multiplied; but this does not seem to me to be inexplicable, as the *Opalinæ*, as is well known, are Entozoa, and their natural conditions of existence could hardly if at all be realized for them during the experiment.

If we accept as possible the faculty of the Infusoria to break up spontaneously and to rise again anew from the ruins, we get for them conditions exactly analogous to those of the Metazoa, as may be shown by an example already mentioned by me:—A worm (e. g. *Nais*) can divide spontaneously into two equivalent individuals, just like an Infusorian; a worm (e. g. *Ctenodrilus monostylos* *) can break up spontaneously

* Zeppelin, "Ueber Bau und die Theilungsvorgänge des *Ctenodrilus monostylos*," in Zeitschr. f. wiss. Zool. Bd. xxxix. (1883).

into irregular fragments, which then gradually become regenerated into perfect animals; we find the same thing in Infusoria (*Opalina*); and, finally, a worm (e. g. *Nais*) may be cut up artificially into pieces which are able to replace the lost parts; and the Infusoria, as already shown, possess the same faculty. The only difference is that in the regeneration of the Metazoa the cells perceptibly perform what in the Protozoa is the function of the elementary parts.

The Significance of the Nucleus in Regeneration.

When once the general fact of the regenerative faculty was established, the next point was to ascertain the behaviour of the nucleus in regeneration, and its influence, if any, thereupon. In the above-mentioned experiments of the botanists upon the multinucleate cells of *Vaucheria* it had been already pretty certainly demonstrated that in artificial division large nucleiferous portions continue capable of living, while small ones without a nucleus perish; nevertheless it could still be objected that perhaps the smallness of the fragment might cause the want of vitality. The following experiment of Nussbaum's is more conclusive as to the indispensability of the nucleus in regeneration:—"In one instance an Oxytrichine was divided in the direction of its length. On microscopic examination it appeared that all the four nuclei had escaped by the cut surfaces. The fragments were enucleate. The smaller one moved for three hours by the retained ciliary action. The larger fragment lived on until the following day, but had not resumed the Oxytrichine form, as had been the case in all the numerous other experiments in nucleiferous fragments. It moved about in the fluid in the form of a short-tailed sphere. On the second day after the artificial division this piece also had perished." "It would therefore appear," says Nussbaum, "that for the preservation of the formative energy of a cell the nucleus is indispensable." Although he did not choose to state this proposition with perfect certainty, this was probably due to the fact that he could only appeal to a single experiment, in which perhaps inappreciable accidents might have come into play; and therefore I tried whether with *Stentor* any further support for the above-mentioned opinion could be obtained. I was myself not *à priori* convinced of it, for I had frequently had occasion to observe apparently unaltered existence in Protozoa which had lost their nucleus; and I have already described my own and some other observations upon this point under the title of "Ueber die Einflüsslosigkeit des Kerns auf die Bewegung, die Ernährung und das Wachsthum einzelliger Thiere," in the 'Biologische Centralblatt' (Band iii. p. 580), and at the end of the article put forward the proposition "that the nucleus has no importance in those functions of the cell-body which do not stand directly in relation to reproduction."

I said expressly all functions which do not stand in relation to reproduction, and, as will appear hereafter, I had in this judged quite correctly: *a further vegetation and even an increase in size is possible, even without a nucleus, under certain circumstances, but a reproduction or regeneration* (i. e. *a new production of parts of the body*) *cannot occur without the intervention of the nucleus.*

Considerable difficulties lay in the way of the experiments with *Stentor*, inasmuch as the necklace-like nucleus traverses the whole body, and it is therefore difficult to separate a part in such a way that it should contain no portion of the nucleus. I first of all tried to cut away small portions of the anterior part of the body, and I succeeded frequently in avoiding any injury to the nucleus in so doing (fig. 8 *a*). After isolating them I found such small pieces on the following day tolerably perfect in form; I stained them with picrocarmine, when it appeared that they actually contained no nuclear constituent (fig. 8 *b*), and I thought I might now conclude from this that a regeneration might occur even without the presence of a nucleus. I was also led to the same conclusion at first by another experiment:—Starting from the fact that the necklace-like nucleus of the Stentors fuses during fission into a bean-shaped mass, I selected individuals which just showed the commencement of division, *i. e.* in which the middle of the body was just beginning to show a new peristome (fig. 9); in one such individual I succeeded in making a transverse section immediately in front of the foundation of the peristome in such a way that the greater part of the mass of the nucleus was caused to escape. The two portions were isolated, and on the following day both had become quite perfect animals. When stained on the slide * it now appeared that one of these two Stentors actually possessed no trace of a nucleus, while the other contained only a small residue of it. In this case also therefore regeneration had apparently occurred without the influence of the nucleus. On more particular investigation, however, both this and the former

* Stentors may very easily be stained on the slide, as, when flooded with absolute alcohol, they usually remain firmly attached to the glass. In this Infusorian particularly the nucleus takes up picrocarmine with extraordinary avidity, and is always stained dark red before the cytoplasm begins to acquire colour.

experiment appeared capable of another interpretation. In the small pieces separated from the anterior end the perfect appearance on the following day was due not to regeneration, but to simple wound-healing, in the course of which the separated portion of the peristomial circlet had closed up into a circle, and thus an illusory picture of a perfect Infusorian was produced; but a new mouth had not been formed when the original one was left behind by the incision; in short, what had been lost was not replaced by anything new (fig. 8 *b*). In the second case also we have not to do with a regeneration, for in the middle part of the body of the Stentor under experiment a new peristomial area with the adoral zone of cilia was already in course of formation, and the incision, which passed close in front of this rudiment, had actually only divided the *Stentor* into two parts, which would soon afterwards have separated spontaneously from each other. In the portion which had retained the original anterior end it was only necessary for the wound to close up and the body to taper again into the form of the hinder end; in the other the wound also closed, and the accompanying rudimentary peristome simply passed through its further course of development until the formation of the perfect peristomial area and the buccal spiral. From these observations therefore it only appears *that a process of wound-healing may occur in Infusoria even without the presence of a nucleus, and that a process of new-formation, when once in progress, may also continue without disturbance without the help of the nucleus*; the impulse thereto, as we shall see hereafter, has indeed proceeded from the nucleus, but, this being once given, the impulsive element may be removed without at the same time cancelling the movement. At least I think that we cannot interpret the second experiment, which I afterwards often repeated in the same way, otherwise than *that in the new-formation of parts in Infusoria we must see a movement which incessantly presses towards its object when it has once been set going. But such a movement cannot be started*, i. e. "*organula*" *cannot be produced anew, when the nucleus is lost.* This is proved with certainty by the experiments which I will now describe. I cut a small piece off a Stentor in such a manner that no part of the peristomial circlet was separated with it, because this might afterwards have led to mistakes, and I isolated it (fig. 10); it was not regenerated, and on subsequent preparation it appeared that no constituent of the nucleus was contained in it. I repeated the experiment, and again separated from another individual a small portion, on which also there was no trace of peristomial cilia (fig. 11); on the next day, however, this piece had become regenerated, and on the application of reagents it proved to be nucleated. Further, I cut a Stentor, in the manner above described, into four pieces (fig. 3); next day three of these pieces (A, B, C) were completely regenerated, one of them (D) not so, and this last when stained proved to be non-nucleate, while the three others had retained portions of the nucleus. The non-nucleate piece, which was incapable of regeneration, was not at all smaller than the others and less endowed with vitality on account of smaller dimensions; but all the four portions were of about the same size, and the non-nucleate piece was even of much larger dimensions than many parts separated in other experiments which were very well regenerated *.

The following experiment is still more conclusive as to the importance of the nucleus in regeneration. If we cut away the posterior extremities from a considerable number of Stentors and isolate these separated parts, which therefore retain no constituents of the peristome, we find them next day in different states—some of them have become regenerated into perfect Stentors with a new peristome, mouth, and œsophagus; in others the regeneration is in progress but not yet quite completed; and, lastly, in a third portion we only find that the wound has closed up, the animals swim about like the rest, but no trace of regeneration is exhibited. When stained on the slide it is seen that the perfectly regenerated pieces contain a normal necklace-like nucleus; that those in which the restoration is delayed have only retained a small fragment of nucleus; and that those which prove to be incapable of regeneration are quite destitute of nucleus. I have frequently kept such non-nucleate pieces alive for several days; but they always perished without the occurrence of any new-formations.

I have also undertaken similar experiments with some other Infusoria, but without further results, as they were all less fitted for the purpose than *Stentor*. On the other hand, I succeeded with *Amœba proteus* in obtaining perfectly good results. As is well known, *Amœba proteus* has only a single, tolerably large nucleus †, and for this reason it is not difficult to divide into a nucleate and a non-nucleate half (fig. 12). If the section be made successfully and the two portions isolated, we see that one of them continues without disturbance to push forward and retract its pseudopodia (A), in short it has undergone no change in its habit; while in the other portion

* I may mention that I several times repeated this and the following experiment in order to insure myself against possible accidents.

† Gruber, "Studien über Amöben," in Zeitschr. f. wiss. Zool. xli.

(B) the pseudopodia disappear, although a feeble flow of protoplasm is at first still visible, and in course of time the fragment dies completely. I divided such an *Amœba* on April 14; on the 16th the one half was as active as at first, but the other had become globular and was in course of perishing: when stained the former proved to be the nucleate and the latter the non-nucleate half; and the same result was furnished by all other experiments *. *Here, therefore, the removal of the nucleus also immediately superinduces an alteration of the mobility, which will not be the case in the Infusoria or probably in most Protozoa*, at least even in Heliozoa I have seen non-nucleate fragments move as freely as the nucleated ones. *But what is superinduced in all Protista, and generally in every cell, by the want of the nucleus is the incapacity to replace lost parts, to produce new structures.*

Thus for the "maintenance of the plastic energy of a cell," as Nussbaum expresses it, the nucleus is, in fact, indispensable; and we may say with Weismann †, that "only under the influence of the nucleus the transformable cell-substance again acquires the full specific type." *By a purely empirical course we are here placed before the incontrovertible fact that the nucleus is the most important and the species-preservative constituent of the cell, and that to it is justly ascribed the highest importance in the processes of fecundation and inheritance*, as has been done of late by many naturalists.

As the directing influence in the increase of cells emanates from the nucleus, it appears wonderful that the nuclear substance is often distributed in more or less numerous fragments in the protoplasm, so that, to a certain extent, instead of a monarch, an oligarchy exists in the cell, which, we might suppose, could easily produce a confusion in the development. Perhaps, to obviate this and also to permit a uniform distribution of the nuclear substance in the daughter-individuals, in most multinucleate Infusoria we observe a preliminary union of the numerous nuclei into *one*. When this amalgamation does not take place during multiplication ‡ we must conceive of all the nuclei of the same

* In his "*Amœba villosa*," Wallich twice observed a spontaneous division without participation of the nucleus, in which the two daughter-individuals behaved in exactly the same way as those artificially produced; whether the non-nucleate portion afterwards perished is not mentioned (see Wallich, "*Amœba villosa*, &c.," in Ann. & Mag. Nat. Hist. ser. 3, vol. xi. p. 444.)

† Weismann, 'Die Continuität des Keimplasmas als Grundlage einer Theorie der Vererbung,' Jena, 1885, p. 29.

‡ According to Bütschli, as is well known, the nuclei do not become amalgamated during fission in *Loxodes rostrum*. I have also always found individuals of this Infusorian which were just engaged in dividing, to be

cell-individual as perfectly congruent in structure and function. Moreover, in most multinucleate Protozoa the nuclei appear to the observer congruent in structure, for there are generally no data from which to demonstrate any differences. For this reason it was interesting to me to find in the two nuclei of *Amœba binucleata* an object which may be investigated from this point of view. As I pointed out in my description of this remarkable *Amœba* *, the nuclei, two of which are present, are very large, and are distinguished by a very variable form and arrangement of the chromatic substance, and it appears that the two nuclei of the same *Amœba* always agree in this respect (fig. 13). For example, if the chromatin is distributed in the nuclear fluid in larger and smaller fragments, this is the case in both nuclei (*a*); if it is broken up into a finely granular mass (*b*), if there is a central nucleolus-like lump in the nucleus (*c*), or if the chromatic substance is deposited towards one side (*d*), the two nuclei always agree with each other. *Here, therefore, we can definitely prove the congruence of the nuclei; and I believe that it also furnishes a proof that the chromatin in the nucleus is an important factor, that something depends upon the kind of its substance, and we have not to do with a mere accumulation of nutritive material.*

It still remains for me to say something about the part which the *subsidiary nucleus* has to play in the regenerative processes; but I am unfortunately not in a position to say anything positive about it.

Until quite recently nothing was known of *subsidiary nuclei* in *Stentor*, and only Maupas † has made any statements regarding them, in which he describes the subsidiary nuclei as separate granules irregularly distributed, one or more of which lie in the neighbourhood of each joint of the nucleus.

Balbiani did not succeed in confirming Maupas's observation, but I have repeatedly been able to convince myself of its correctness. Very frequently, although not always, my preparations showed, coloured red with carmine, corpuscles agreeing with those described by Maupas, as I could perceive

multinucleate. Nevertheless the fusion and subsequent reseparation into numerous nuclei might have already taken place before the commencement of division became visible on the body of the Infusorian, just as I have described in the case of *Oxytricha scutellum* (Gruber, "Ueber Kern und Kerntheilung bei den Protozoen," in Zeitschr. f. wiss. Zool. Bd. xl.).

* "Studien über Amöben," &c.

† Maupas, "Contributions à l'étude morphologique et anatomique des Infusoires ciliées," in Arch. de Zool. Exp. et Gén. sér. 2, tome i. pp. 652 *et seqq.*

from the drawings which that naturalist was kind enough to send me. Notwithstanding the small size and often very irregular distribution of these structures, it seems to me very probable that they must be regarded as subsidiary nuclei. But this can be said with perfect certainty only when we have succeeded in tracing their behaviour during the division and conjugation of the Stentors. As regards regeneration, I could discover no sort of influence that they were able to exert upon that process.

Observations upon the Spontaneous Division of the Infusoria.

So far as I know, no experiments have been made in order to ascertain whether in the multiplication of the Protozoa by division there exists any regularity as to the time in which the divisions follow one another, whether a definite number of divisions occur between two periods of conjugation, whether the occurrence of division is caused by increased nourishment and the growth consequent upon this, or, on the contrary, by unfavourable external circumstances, or whether it is not at all the consequence of external impulse, but is governed and produced by internal causes. These and many other questions are still unanswered, and even the experiments which have been undertaken for their solution have at present made only a slight commencement, so that they can make no claim to completeness, seeing that they have not furnished the opportunity of completing them by more perfect ones. *Stentor cæruleus* again served me as my chief object of experiment; I had it in abundance at my disposal, and from its considerable size it could be easily isolated and watched.

One series of experiments consisted in isolating Stentors, and indeed, if possible, such as were just about to divide; when the division took place the daughter-individuals were separated and observed by themselves, in order to see how and when their division into grandchildren took place. It appeared *that this took place, in most cases, simultaneously in the daughter-individuals*, although they were kept in separate glasses. By "simultaneously," however, I do not mean that the division takes place in both Infusoria at the same moment, but perhaps within an hour or in the course of a few hours, but at any rate on the same observation-day, which I will reckon as from 9 A.M. to 4 P.M. Very often also the isolated individuals divided during the night, and in the morning I found both daughters divided into two granddaughters. The small differences of time which occur between the divisions of the daughter-individuals are of course increased in the following generations, so that in the case of the granddaughters and great-granddaughters of a common ancestral individual we can no longer speak of simultaneous division; in these differences of many hours and even of days occur. As the same share of nuclear substance falls to the two congruent halves into which the Infusorian breaks up in division, and indeed, as we assume, to each of them one of the morphologically and physiologically equivalent halves of the original nucleus, it might be supposed that under the same external conditions therefore (for example, those of common residence in a very small quantity of water) the daughter-nuclei must be absolutely alike in the exertion of their dominion over the plasma, and therefore in their influence upon division, so that in the daughter-individuals increase will take place at the same moment. Why small differences should occur I cannot at present say definitely; I believe, however, that in this circumstance we may see an indication *that the morphological and physiological congruency of the two daughter-individuals produced by division is by no means quite an absolute one.*

I may remark further that in other Infusoria, such as *Clymacostomum*, *Stylonychia*, and *Paramæcium*, I have also been able to prove the (nearly) simultaneous multiplication of the daughters of the same individual.

As regards the time that intervenes between the different divisions, I can only say anything definite in relation to *Stentor*, as this, hitherto, is the only Infusorian in which I have succeeded in making a number of observations upon this point. Singularly enough it appeared that *division took place in most cases at intervals of two days*, that daughter-individuals divide into granddaughters on the second day after their separation, and granddaughters in another two days into great-granddaughters, and so forth. *In forty-two out of fifty-six cases division took place always on the second day after the preceding one*; six divided as early as the next day, five only on the third, and three after four, five, or more days. In *Stentor cæruleus* therefore we may almost regard it as the rule that the above-mentioned interval of time is maintained between each two divisions. The question now, however, is whether this phenomenon is normal, or whether it is called forth by unnatural conditions of existence. This is difficult to decide, as these investigations can only be made on isolated animals, and therefore on animals living in small quantities of water. But even if we suppose that the small quantity of water has produced the tendency to rapid division, this would only lead to the assumption that this tendency may also occur in nature when from any circumstances the pool, brook, &c. was nearly dried up; the regularity with which the divisions follow one another in time is not thus explained, and *this can apparently only be the expression of a constantly acting internal law.*

The absence or presence of nutritive material for the Stentors was in all these experiments without influence upon the time of the division. I had isolated animals in watch-glasses containing nearly pure water, and in others in which the water swarmed with *Paramœcia* (a chief food of the Stentors) and other Infusoria; but in both the multiplication went on in the same manner, and indeed always so that the animals did not grow between two divisions, and therefore lost in volume from one division to the next. I have frequently made measurements of the individuals under experiment before isolation, measuring them while swimming about, when they present a mean state of extension; then the daughters, granddaughters, &c. were also measured, and it was found that the volume decreased to about one half, then to a quarter, and so forth. I say about, because the animals produced by division appeared a little larger than the corresponding fragments, which may be due probably to inception of water. The Stentors which I isolated were generally nearly of the same size, and they divided only to the third generation, so that the last generations in these experiments were always nearly of the same dimensions.

If I isolated smaller animals, they divided only to the second generation, which also again showed the smallest measurement.

I believe there is no doubt that in these phenomena a normality is expressed, and that we have not to do with the products of accident. Even in the aquaria in which the colonies of *Stentor* live under natural conditions of existence we often find the Infusoria of very small average size, and it may very well be that these had just been subjected to a rapid sequence of divisions. *I believe that among the Infusoria we may distinguish two kinds of spontaneous division, one of which occurs when the individual by growth has attained a certain size which cannot be exceeded*; this is the multiplication which has been characterized as the growth of the individual beyond the prescribed measurement. *A second mode of increase is by divisions following upon one another rapidly and in definite intervals of time without intervening growth, and therefore combined with continual decrease of the size of the body down to a definitive smallest measurement.* This latter mode of multiplication, of the existence of which I have already furnished proof, will occur when the Infusoria are placed under unfavourable conditions, and it seems desirable, for the preservation of the species, to produce a great number of individuals very rapidly. At the close of these hurried divisions a period of conjugation would then occur, and this, as is well known, has always been observed in very small individuals. If the last-mentioned mode of increase were the sole one, we should always observe in every colony of Infusoria a diminution of the individuals combined with an increase of their number, and an equally regular cyclical recurrence of the period of conjugation. But every one who has been long occupied with the Infusoria knows that this is not the case; and it is particularly well known to those who have for a long time observed colonies rich in individuals and constantly increasing, and sought in vain for conjugation-states, which at other times had been present in great numbers.

I must not, however, develop these ideas any further, for, as already remarked, the empirical facts from which they proceed still stand on too weak a foundation, and I will rather wait until time and opportunity may furnish me with suitable material for working further upon them.

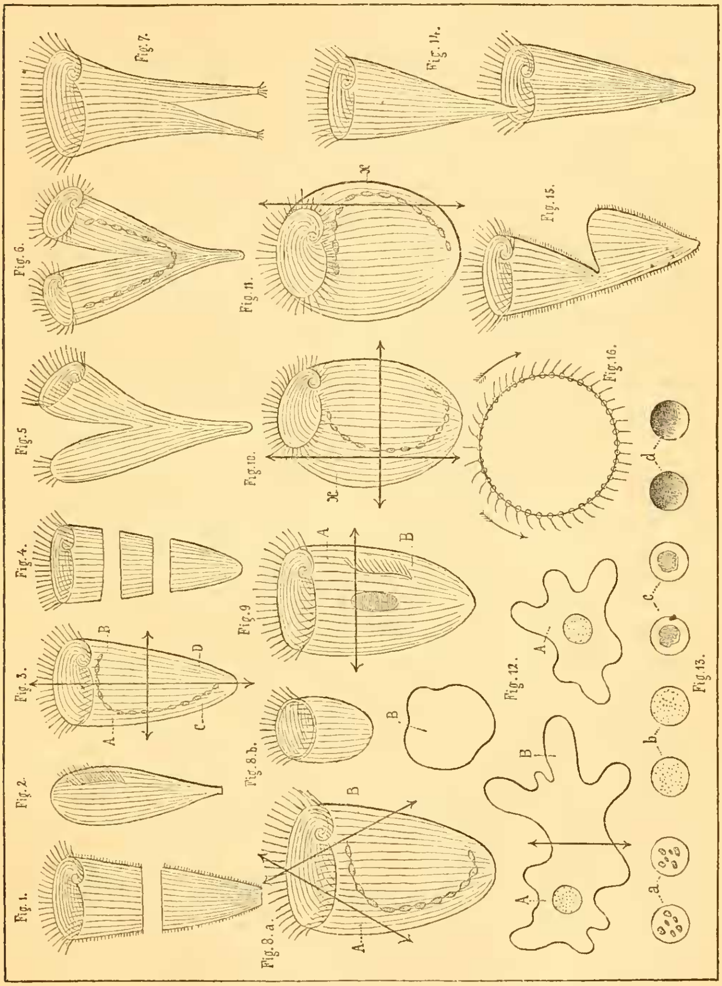
On the Nervous System of the Infusoria.

In my experiments with Stentors my attention has been called to a question which I would here briefly touch upon, namely, what may be the nature of the nervous elements in the cell-body of the Infusoria? Some light is thrown upon this by the behaviour of the Infusoria during conjugation and spontaneous division, as I have already indicated in my preliminary communication above referred to. Thus if we observe a pair *in copulâ* or an Infusorian engaged in division in which the two halves are not yet completely separated, one is struck with the fact that these animals move exactly like a single individual, *that both of them make exactly concordant movements so long as they are still united by a bridge of protoplasm.* I have frequently traced this in different species of Infusoria; but here, again, the Stentors are particularly adapted to the purpose, as the movements are so distinctly to be detected under the microscope in the great peristomial cilia. So long as the two daughter-individuals are united by even the thinnest thread of protoplasm (fig. 14) they behave precisely like a single individual; if the peristomial cilia of the anterior half strike forward, so also do those of the posterior, and at the same moment in which the former, from any cause, change the direction of their motion, so also do the latter. The natation is therefore perfectly uniform, and the two animals glide quietly through sand-grains, filaments of algæ, &c., one after the other. But if the anterior one meets with an obstacle, stops and swims backwards, the posterior Infusorian does this also at the same time. It is therefore not as if the second individual simply followed the first, and if the first can go no further the second would still endeavour for a time to swim forward until it is held back. If one of the halves shrinks together in consequence of an unpleasant contact, the other does so also at the same moment; in short all the movements are perfectly synchronous until the last uniting threadlet is ruptured between the two individuals, each of which then swims away in a different direction. The same result is obtained if we succeed in making a transverse incision in a Stentor in such a manner as to produce two halves, which, as in spontaneous division, are united by a narrow bridge of protoplasm (fig. 15). Then also these two loosely connected pieces move quite uniformly, and one of them does not attempt to swim backwards while the other steers forwards. As in this case the posterior half lacks the peristome, the simultaneous movements are performed by the body-cilia. If then, as these observations show, a very slender and even thread-like bridge of protoplasm suffices to cause the loosely connected pieces to behave as one physiological individual, this proves that *the nervous functions in the Infusorial body are not confined to definite courses, and that the exertion of will uniformly governs every protoplasm-element.* Consequently no circumscribed central organ can be present; but every plasmatic particle is a central organ and conductor in one, i. e. *the nervous potency of the cell is diffused.* This does not render it impossible that at the same time *threads* of nervous nature may exist, as for example in the case of the innervation of cilia which have to beat at unequal times, as Engelmann believes he observed to be the case in *Stylonychia**.

This assumption also explains how it is possible that swimming colonies of Protozoa are able to perform movements in accordance with a purpose. For example, if we observe a *Volvox*-sphere, which may consist of many hundred individuals, we see that in its movements it behaves no otherwise than as a holotrichous Infusorian; the sphere swims forwards and backwards, turns in a circle, remains still when necessary, according as some obstacle stands in its way or the course is free. But as the individuals are situated on the surface of a sphere they cannot all strike in the same direction with their flagella, but the movements of these must compensate

* "Zur Anatomie und Physiologie der Flimmerzellen," in Pflüger's Arch. für Physiol. xxii. (1880) p. 505.

each other; and in a colony swimming straight forwards we see those on the left side striking to the left, and the others to the right, so that a current glides along the left side and another along the right side of the sphere (fig. 16), as has been already indicated by Ehrenberg by arrows in one of his



figures*. Thus all the individuals of the colony are governed by a common will which is diffusedly inherent in the protoplasm, and which can only embrace all the members of the colony in this way, *because the latter are united to each other*

* Die Infusionsthierchen, 1838, Atlas.

by cords of protoplasm. I am convinced that these bridges serve much more for the establishment of a nervous unity than for the reciprocal nourishment of the individual animals.

In the higher Protozoa, and therefore in the Infusoria, it seems to me probable that *the seat of the diffused nervous potency is chiefly to be sought in the cortex.* Thus certainly this and not the parenchyma is alone capable of delicate sensibility, otherwise the frequently observed inception of inordinately large nutritive bodies must be attended by painful sensations. Further, this swallowing of bodies which extend and rupture the Infusoria shows us at once that we need not expect to find in the parenchyma any differentiation into special organula, fibres, &c. I once made a very instructive observation in this direction upon a *Clymacostomum virens.* This Infusorian had swallowed a single wheel-animalcule, which was rushing about in the parenchyma as if mad, stirring up everything, and sometimes pushing out the cortical zone, sometimes drawing it in by means of its rotatory organ. The *Clymacostomum*, however, seemed to be no further troubled by this riotous guest in its interior, for it swam about in the water quite quietly and uniformly. But while other animal prey, such as small holotrichous Infusoria, which were frequently devoured by the same individual, were digested in a short time (about a quarter of an hour), the wheel-animalcule remained alive for twenty-four hours; it lay quiet indeed, but the rotatory organ was still in motion. Of course, in such a long time it must have produced sad ravages in the body of the Infusorian if any complex structures had been present therein. But the only thing observable in the *Clymacostomum*, which was still very lively, was that at the posterior extremity, where the wheel-animalcule lay, the body was somewhat indented; but this had disappeared on the following day when the animalcule had died and been digested.

Let no one who is paying attention to the Protozoa omit seizing upon such chance observations as the above, for it is by them we shall most readily get to understand when and how the vital phenomena are performed in the simple but enigmatical protoplasmic body of the "unicellular animals."

XLV.—*On a new Species of* Psilotites *from the Lanarkshire Coal-field.* By Robert Kidston, F.G.S.

Psilotites unilateralis, Kidston, n. sp.

Description. Stems narrow, irregularly striated, provided with a lateral row of thorn-like projections.

Remarks. The specimen shows portions of three stems